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EPIDEMIOLOGY AND SURVEILLANCE

Comparison of Commensal and Clinical Isolates for Diversity of Plasmids in *Escherichia coli* and *Klebsiella pneumoniae*

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ABSTRACT In this study, the plasmid content of clinical and commensal strains was analyzed and compared. The replicon profile was similar in both populations, except for L, M, A/C, and N (detected only in clinical strains) and HI1 (only in commensal strains). Although I1 and F were the most frequent replicons, only IncI1, sequence type 12 (ST12) was associated with bla_{CMY-2} in both populations. In contrast, the widespread resistant IncF plasmids were not linked to a single epidemic plasmid.

KEYWORDS pMLST, replicon, antimicrobial resistance, *Enterobacteriaceae*, plasmid epidemiology

The most prevalent mechanism in antimicrobial resistance gene (ARG) acquisition by bacterial pathogens is horizontal gene transfer by plasmids (1, 2). PCR-based replicon typing (PBRT) based on plasmid incompatibility (Inc) is currently the standard method for plasmid identification (3, 4). Plasmid multilocus sequence typing (pMLST) schemes allow researchers to differentiate between plasmids within incompatibility groups and to define epidemiological and evolutionary relatedness (5–10) (http://pubmlst.org/plasmid/).

Several plasmids carrying ARGs have been characterized, most of them recovered from clinically relevant bacteria (11–14). In contrast, there is limited information on plasmids in the commensal microbiota of healthy humans without a selection bias for antimicrobial-resistant bacteria. In this scenario, the aim of this study was to provide a better understanding of resistant plasmid diffusion in a clinical context by comparing plasmids within *Escherichia coli* and *Klebsiella pneumoniae* strains isolated from healthy human feces and patients with bloodstream infection.

One hundred and fifty fecal samples were collected during 2014 and 2015 from healthy humans who did not consume antibiotics and were not hospitalized. A total of 145 *E. coli* strains and 12 *K. pneumoniae* strains were isolated. In addition, 202 strains from blood cultures, 99 *E. coli* strains and 103 *K. pneumoniae* strains, from three hospitals in Barcelona were analyzed (one per patient). (The study was approved by the Hospital de la Santa Creu i Sant Pau ethics committee [13/051/1439].) All strains underwent antimicrobial susceptibility testing using disk diffusion (see Table S1 in the supplemental material), and the results were interpreted according to CLSI guidelines (15). The characterization of extended-spectrum β-lactamases (ESBLs), AmpCs, and carbapenemases (16–20) detected in both populations is shown in Table 1. The prevalence of ESBL-producing *E. coli* in healthy carriers (4.7%) was higher than that in

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TABLE 1 ESBL, AmpC, and carbapenemase genes detected in *E. coli* and *K. pneumoniae* from fecal and blood samples

	Gene (no.) detected in:			
	E. coli ($n = 244$)		*K. pneumoniae* ($n = 115$)	
Gene type (no.)	Fecal samples ($n = 13/145$)[a]	Blood cultures ($n = 19/99$)[b]	Fecal samples ($n = 0/12$)	Blood cultures ($n = 17/103$)[c]
ESBLs (43)	$n = 8$; 5.5% $bla_{CTX\text{-}M\text{-}15}$ (4) $bla_{CTX\text{-}M\text{-}14}$ (3) $bla_{CTX\text{-}M\text{-}27}$ (1) $bla_{SHV\text{-}12}$ (1)	$n = 17$; 17.2% $bla_{CTX\text{-}M\text{-}15}$ (10) $bla_{CTX\text{-}M\text{-}14}$ (2) $bla_{CTX\text{-}M\text{-}27}$ (2) $bla_{CTX\text{-}M\text{-}32}$ (1) $bla_{SHV\text{-}12}$ (2)		$n = 17$; 16.5% $bla_{CTX\text{-}M\text{-}15}$ (9) $bla_{CTX\text{-}M\text{-}14}$ (2) $bla_{SHV\text{-}28}$ (5) $bla_{SHV\text{-}2}$ (1)
AmpCs (11)	$n = 5$; 3.5% $bla_{CMY\text{-}2}$ (5)	$n = 5$; 5.0% $bla_{CMY\text{-}2}$ (4) $bla_{DHA\text{-}1}$ (1)		$n = 1$; 1.0% $bla_{DHA\text{-}1}$ (1)
Carbapenemases (2)				$n = 2$; 1.9% $bla_{KPC\text{-}3}$ (2)
Total genes (56)	14	22	0	20

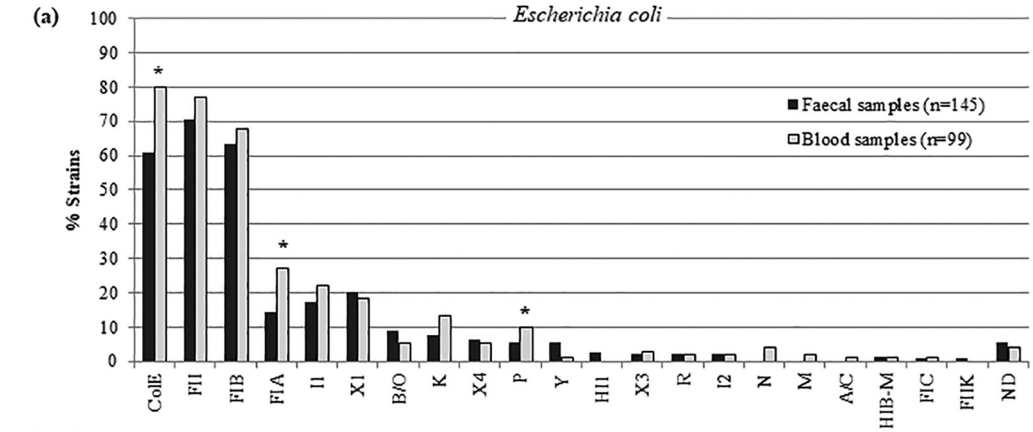
[a]One *E. coli* strain had $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}14}$.
[b]Three *E. coli* strains had $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}32}$, $bla_{CTX\text{-}M\text{-}27}$ and $bla_{DHA\text{-}1}$, and $bla_{SHV\text{-}12}$ and $bla_{CMY\text{-}2}$.
[c]One *K. pneumoniae* strain had $bla_{CTX\text{-}M\text{-}15}$ and $bla_{CTX\text{-}M\text{-}14}$, and two strains had $bla_{CTX\text{-}M\text{-}15}$ and $bla_{SHV\text{-}28}$.

a previous study in Barcelona in 2005 (3.3%) (21) but still within the 3 to 6% average of Europe (22).

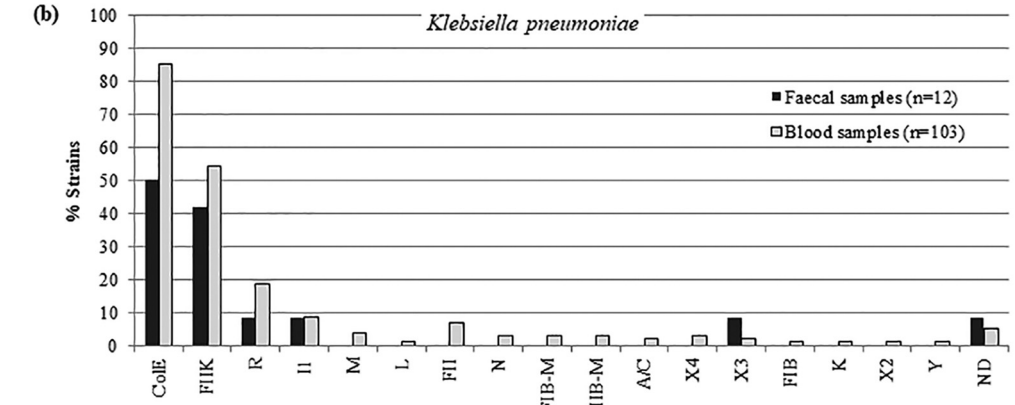
Plasmid identification was performed using the PBRT-kit (Diatheva) and simplex PCR for ColE, X3, X4, L, and M replicons (23–25). Twenty-nine replicons were analyzed, and only FIIS, W, T, U, and HI2 were not detected in any strain. A total of 978 replicons were identified in the 359 studied strains; 84.1% (302/359) harbored from one to four replicons, and 10.9% (39/359) harbored from five to seven. In 5% (18/359) of the strains, no replicon was detected. Overall, the results suggest that the replicon content of *E. coli* strains followed a similar trend in patients and healthy individuals, and the most prevalent in both sample groups were ColE, FII, and FIB (Fig. 1A). Nevertheless, replicons M, A/C, and N were only detected in clinical strains in accordance with the literature (26, 27), while FIIK and HI1 were observed only in fecal strains (Fig. 1A). Hence, it might be hypothesized that the hospital environment, where there is high antimicrobial use and intense interhuman transmission, selects for plasmids more adapted to these settings. The plasmid content in *K. pneumoniae* isolates seems to follow similar trends (Fig. 1B) to that of *E. coli*, but this could not be confirmed due to the low number of strains obtained from fecal samples. Notably, both the diversity and frequency of replicons were higher in *E. coli* than in *K. pneumoniae*, except for those of R and FIIK (Fig. 1C).

IncF and IncI1 plasmids have been reported in *Enterobacteriaceae* as promoters of beta-lactamase gene dissemination in multiple environments, specifically $bla_{CTXM\text{-}15}$ and $bla_{CMY\text{-}2}$ (13, 28–32). In this study, 56 beta-lactamase genes were detected (Table 1). After S1 pulsed-field gel electrophoresis (PFGE) and Southern hybridization (19, 33), 75% of ESBL, AmpC, and carbapenemase genes identified in *E. coli* (27/36) and *K. pneumoniae* (15/20) were located on a plasmid, the most prevalent being IncF and IncI1 (37% for both) in *E. coli* and IncF (47%) and IncR (20%) in *K. pneumoniae*. The predominant genes were $bla_{CTX\text{-}M\text{-}15/14/27}$ and $bla_{CMY\text{-}2}$ in IncF and InI1 plasmids of *E. coli* (see Fig. S1A in the supplemental material) and $bla_{CTX\text{-}M\text{-}15}$ in IncF plasmids of *K. pneumoniae* (see Fig. S1B). Figure 2 summarizes the 49 ESBL-, AmpC-, and/or carbapenemase-producing strains detected in the study, the plasmids they harbored, and the location of the beta-lactamase genes.

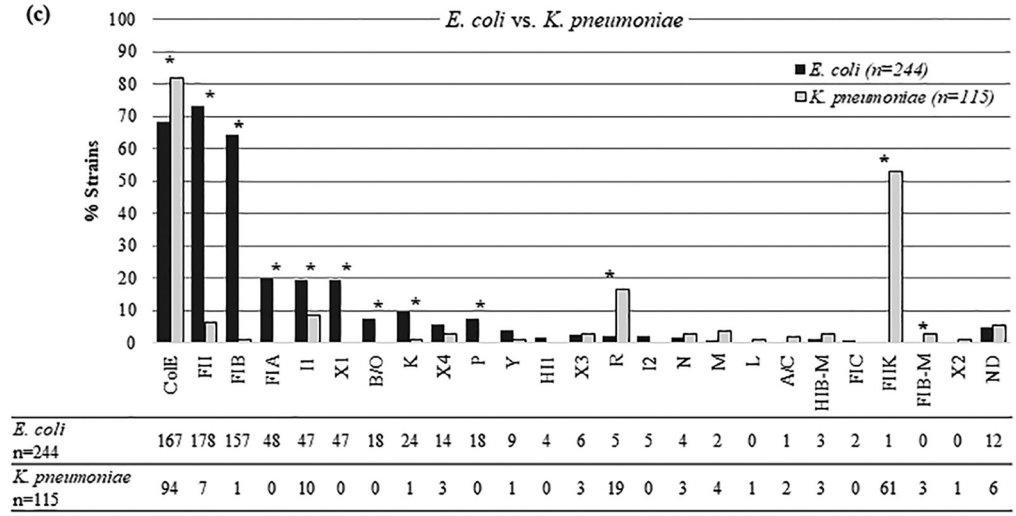
As IncF and IncI1 were two of the most frequently detected plasmids in both fecal and clinical samples (27, 34, 35), they were further characterized using the pMLST method (5, 10). In *E. coli* strains, 29 different IncI1 sequence types (STs) were detected, 59% of which were assigned as new STs. This result reflects the great diversity within this plasmid family, with only ST12 and ST36 being present in both clinical and fecal populations (see Fig. S2 in the supplemental material). Moreover, some of the most



	ColE	FII	FIB	FIA	I1	X1	B/O	K	X4	P	Y	H11	X3	R	I2	N	M	A/C	HIB-M	FIC	FIIK	ND
Faecal samples n=145	88	102	92	21	25	29	13	11	9	8	8	4	3	3	3	0	0	0	2	1	1	8
Blood samples n=99	79	76	67	27	22	18	5	13	5	10	1	0	3	2	2	4	2	1	1	1	0	4



	ColE	FIIK	R	I1	M	L	FII	N	FIB-M	HIB-M	A/C	X4	X3	FIB	K	X2	Y	ND
Faecal samples n=12	6	5	1	1	0	0	0	0	0	0	1	0	1	0	0	0	0	1
Blood samples n=103	88	56	18	9	3	4	1	3	3	3	2	3	2	1	1	1	1	5



	ColE	FII	FIB	FIA	I1	X1	B/O	K	X4	P	Y	H11	X3	R	I2	N	M	L	A/C	HIB-M	FIC	FIIK	FIB-M	X2	ND
E. coli n=244	167	178	157	48	47	47	18	24	14	18	9	4	6	5	5	4	2	0	1	3	2	1	0	0	12
K. pneumoniae n=115	94	7	1	0	10	0	0	1	3	0	1	0	3	19	0	3	4	1	2	3	0	61	3	1	6

FIG 1 Replicon prevalence. (a) Comparison between replicons detected in *E. coli* from clinical and fecal samples. (b) Comparison between replicons detected in *K. pneumoniae* from clinical and fecal samples. (c) Comparison between replicons detected in *E. coli* and *K. pneumoniae* from the total strains. Tables show the number of each replicon detected. ND, not detected. *, Statistical differences ($P < 0.05$) between each population, fecal and blood samples or *E. coli* and *K. pneumoniae*.

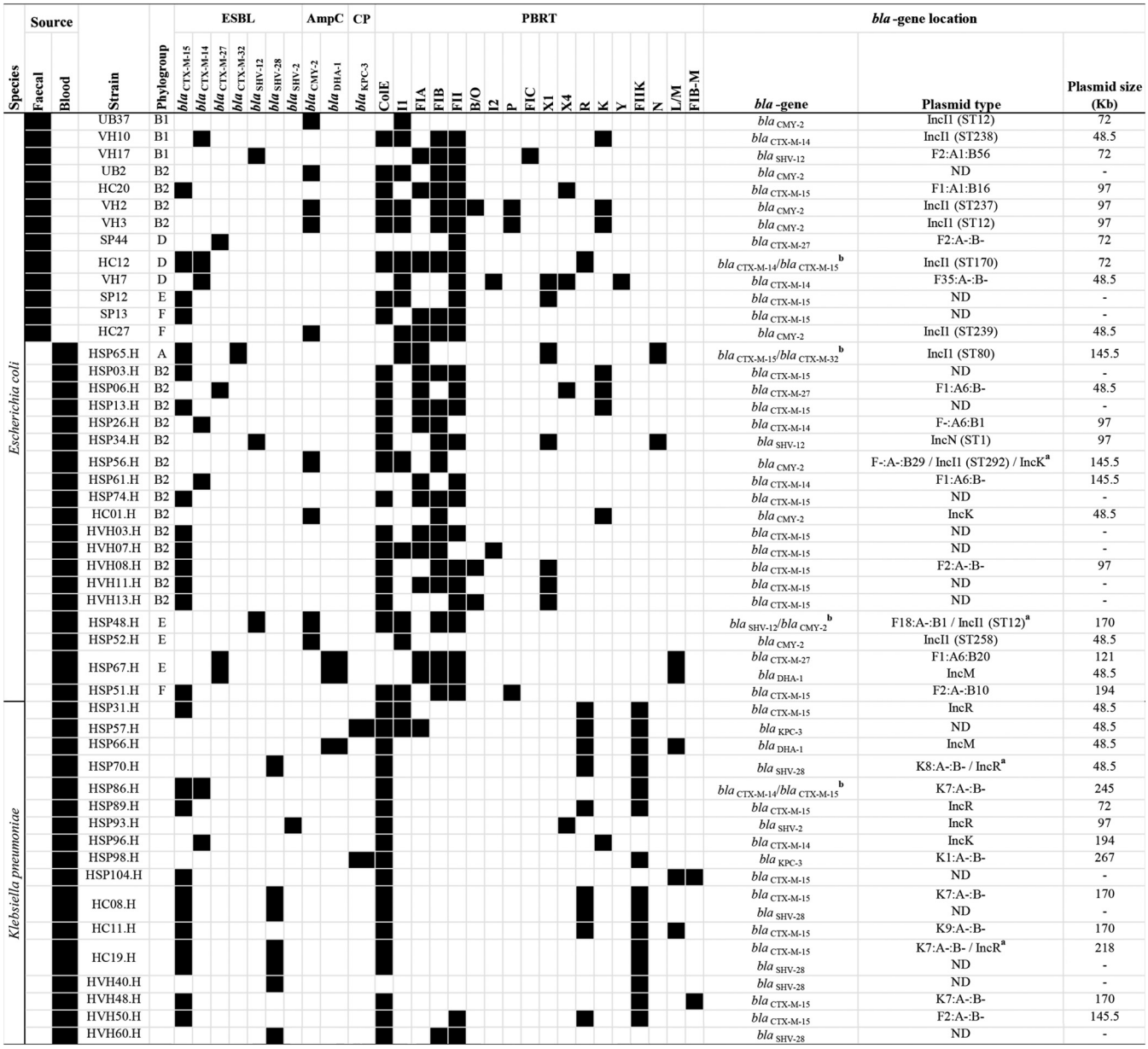


FIG 2 Heat-map summary of the sources, phylogenetic groups, β-lactamase resistance genes, and the corresponding Inc plasmid types and their sizes for 49 β-lactam-resistant *E. coli* ($n = 32$) and *K. pneumoniae* ($n = 17$) strains from fecal and blood samples. Black and white squares denote the presence and absence of a particular feature, respectively. ND, not determined; CP, carbapenemases. [a], Plasmids where the replicon hybridization occurred in the same plasmid size (hybrid plasmids). [b], *bla* Genes detected in the same plasmid.

frequently reported STs worldwide (ST2, ST12, ST26, and ST36) (36) were only found in *E. coli* from healthy humans. The detection of many newly assigned STs in the clinical isolates and a scarce number of the most reported STs suggest that the latter may have been overreported due to their involvement in ARG dissemination, resulting in an epidemiological bias.

In addition, IncI1 plasmids have been associated with the carriage of bla_{CMY-2}, particularly IncI1 ST2, ST12, and ST23 (5, 36–38). In the current study, all identified ST12 plasmids harbored bla_{CMY-2} and were detected in *E. coli* from both populations (Fig. 2). These results support the suggestion that some IncI1 plasmids have been able to evolve and persist in clinical settings thanks to particular features that provide resistance, persistence, and adaptive success, which would explain why they are more frequently reported and described as epidemic plasmids (36–38).

TABLE 2 Phylogenetic groups detected in *E. coli* strains isolated from fecal and blood samples

E. coli isolate type[a]	No. (%) detected of each phylogenetic group									
	Total no.	A	B1	B2	C	D	E	F	Clade I	Unknown
Total *E. coli* isolates	244	26 (10.6)	22 (9)	124 (50.8)	3 (1.2)	32 (13.1)	15 (6.1)	17 (6.9)	4 (1.6)	1 (0.41)
Fecal samples	145	23 (15.9)	11 (7.6)	59 (40.7)	3 (2.1)	23 (15.9)	8 (5.5)	13 (8.9)	4 (2.7)	1 (0.7)
Susceptible	71	16 (22.5)	1 (1.4)	24 (33.8)	3 (4.2)	10 (14.1)	7 (9.9)	9 (12.6)	1 (1.4)	0
Resistant	74	7 (9.5)	10 (13.5)	35 (47.3)	0	13 (17.6)	1 (1.3)	4 (5.4)	3 (4.1)	1 (1.3)
Blood samples	99	3 (3)[b]	11 (11.1)	65 (65.7)[c]	0	9 (9.1)	7 (7.1)	4 (4)	0	0
Susceptible	9	0	1 (11.1)	6 (66.7)	0	2 (22.2)	0	0	0	0
Resistant	90	3 (3.4)	10 (11.1)	59 (65.5)[d]	0	7 (7.8)	7 (7.8)	4 (4.4)	0	0

[a]Susceptible indicates susceptibility to all antimicrobials tested. Resistant indicates resistance to at least one of the antimicrobials tested.
[b]Statistical differences ($P = 0.002$) between A phylogroup strains from fecal and blood samples.
[c]Statistical differences ($P < 0.001$) between B2 phylogroup strains from fecal and blood samples.
[d]Statistical differences ($P < 0.001$) between resistant B2 phylogroup strains from fecal and blood samples.

After defining the final number of IncF plasmids ($n = 279$; 211 in *E. coli* strains and 68 in *K. pneumoniae* strains) by Southern hybridization of F replicons within each strain, subtypification using replicon sequence typing (RST) was performed. In *E. coli* strains, 86 different FAB (FII, FIA, FIB) formulas (indicating the allele type and number identified for each replicon according to RST scheme [10]) from 205 typeable plasmids (45 from fecal samples, 24 from blood, and 17 from both) were defined, where F29:A−:B10, F2:A−:B1, F2:A−:B−, and F24:A−:B1 were the most frequent (see Fig. S3 in the supplemental material). Some of these formulas have been previously identified in different environments, such as avian-pathogenic *E. coli* strains and uropathogenic and extraintestinal pathogenic *E. coli* strains (27, 39), indicating a broad distribution. In *K. pneumoniae*, 16 different FAB formulas from 68 typeable plasmids (12 from blood and 4 from both) were detected, with K1:A−:B− being the most frequent (see Fig. S3).

IncF plasmids, including F2:A−:B−, F2:A1:B−, F31:A4:B1, and F1:A2:B20, have been associated with the worldwide emergence of CTX-M-15 (10, 12, 40–42). Although these four plasmids were detected in our study, only F2:A−:B− harbored the $bla_{\text{CTX-M-15}}$ gene in a clinical strain and $bla_{\text{CTX-M-27}}$ in a fecal strain. All of the other CTX-M-encoding genes were located in different IncF plasmids (Fig. 2). Thus, according to our results and in agreement with those of previous reports (32, 34), there is no evidence for the persistence of a unique IncF. These highly versatile plasmids are able to adapt to intracellular environments by the rapid evolution of replicon regulatory sequences (10), and they were widely distributed in the *Enterobacteriaceae* before antimicrobial use, facilitating the persistence and spread of beta-lactamases (10, 32). Their coexistence with other resistance determinants also contributes to the dissemination of IncF-CTX-M plasmids (43).

Additionally, *E. coli* strains were assigned to phylogenetic groups following the procedure of Clermont et al. (44) (Table 2), and the presence of 15 virulence factors (VFs) was determined (45, 46) (Fig. 3). In commensal *E. coli*, the prevalence of phylogenetic groups varies among studies (35, 47). It has been reported that the highly diverse hosts and environmental factors, the determinants of virulence, and the antimicrobial pressure can modify prevalence for a better adaptation to commensal habitats (47). In our study, even though commensal *E. coli* presented a higher diversity of phylogroups compared to that of the clinical samples (Table 2), a predominance of the phylogroup B2 carrying high rates of VFs was found in both populations. Although no evident association has been reported between plasmids and phylogroups (34), our results indicate a possible association of HI1 plasmids with phylogroup A ($P \leq 0.007$, Bonferroni's correction was applied).

All VFs studied were detected in both populations. As expected, the clinical strains had a higher diversity (9 to 15 VFs) and frequency of VFs compared to those of the fecal samples (1 to 8 VFs) (Fig. 3). Finally, as supported by other authors (48), an association between some VFs (*fyuA*, *iutaA*, *hlyF*, *iss*, and *traT*) and strains carrying F plasmids was determined ($P \leq 0.003$, Bonferroni's correction was applied).

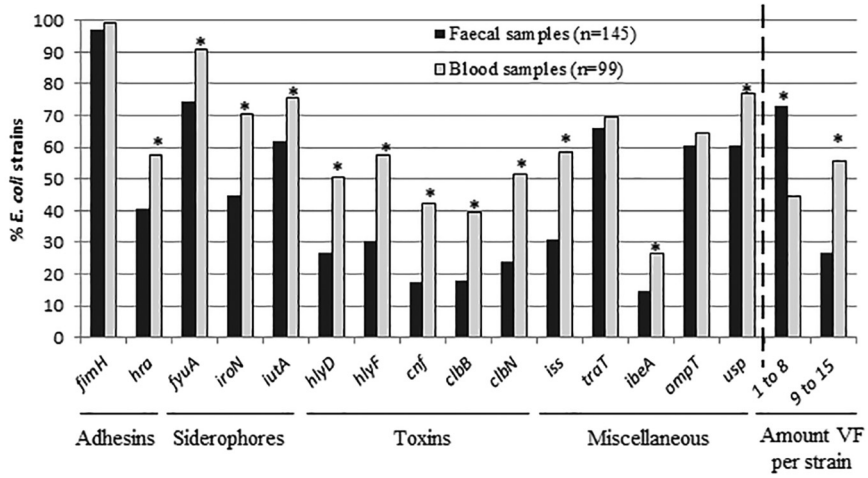


FIG 3 Frequency of virulence factors (VFs) analyzed in *E. coli* from fecal and blood sample strains and percentages of *E. coli* strains according to the number of VFs they carry. Adhesins include *fimH* (mannose-specific adhesin of type 1 fimbriae) and *hra* (heat-resistant agglutinin); siderophores include *fyuA* (yersiniabactin), *iutA* (aerobactin), and *iroN* (salmochelin receptor); toxins include *hlyD* (α-hemolysin), *hlyF* (hemolysin F), *cnf1* (cytotoxic necrotizing factor 1), and *clbB* and *clbN* (colibactin); and the miscellaneous VF genes include *iss* (surface exclusion serum survival protein), *traT* (serum resistance), *ompT* (outer membrane protease), *ibeA* (invasion of brain endothelium), and *usp* (uropathogenic-specific protein). *, Statistical differences ($P < 0.05$) between each population, fecal and blood samples.

In conclusion, new information is provided about the plasmid background in strains isolated in a nonhospital setting. Although a similar trend was observed in the Inc groups from both populations, IncL/M, IncA/C, and IncN plasmids were only detected in clinical strains, whereas HI1 was only present in fecal strains. Also, two different evolutionary pathways followed by plasmids were observed as follows: specific IncI1 plasmids, such as IncI1 ST12, seem to have evolved by acquiring persistence, adaptive, and antibiotic resistance features relevant in clinical settings, whereas the more widespread multireplicon IncF plasmids have randomly acquired resistance genes. Additionally, the findings from this study confirm that strains from healthy individuals have less antimicrobial resistance and fewer VFs and display a higher diversity of phylogenetic lineages (in *E. coli*) than strains causing infection.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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REFERENCES

1. Wright GD. 2011. Molecular mechanisms of antibiotic resistance. Chem Commun (Camb) 47:4055–4061. https://doi.org/10.1039/c0cc05111j.
2. Carattoli A. 2013. Plasmids and the spread of resistance. Int J Med Microbiol 303:298–304. https://doi.org/10.1016/j.ijmm.2013.02.001.
3. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. 2005. Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219–228. https://doi.org/10.1016/j.mimet.2005.03.018.
4. Carattoli A. 2011. Plasmids in Gram negatives: molecular typing of resistance plasmids. Int J Med Microbiol 301:654–658. https://doi.org/10.1016/j.ijmm.2011.09.003.
5. García-Fernández A, Chiaretto G, Bertini A, Villa L, Fortini D, Ricci A, Carattoli A. 2008. Multilocus sequence typing of IncI1 plasmids carrying extended-spectrum β-lactamases in *Escherichia coli* and *Salmonella* of human and animal origin. J Antimicrob Chemother 61:1229–1233. https://doi.org/10.1093/jac/dkn131.
6. Hancock SJ, Phan MD, Peters KM, Forde BM, Chong TM, Yin WF, Chan KG,

Paterson DL, Walsh TR, Beatson SA, Schembri MA. 2017. Identification of IncA/C plasmid replication and maintenance genes and development of a plasmid multilocus sequence typing scheme. Antimicrob Agents Chemother 61:e01740-16. https://doi.org/10.1128/AAC.01740-16.

7. García-Fernández A, Carattoli A. 2010. Plasmid double locus sequence typing for IncHI2 plasmids, a subtyping scheme for the characterization of IncHI2 plasmids carrying extended-spectrum β-lactamase and quinolone resistance genes. J Antimicrob Chemother 65:1155–1161. https://doi.org/10.1093/jac/dkq101.
8. García-Fernández A, Villa L, Moodley A, Hasman H, Miriagou V, Guardabassi L, Carattoli A. 2011. Multilocus sequence typing of IncN plasmids. J Antimicrob Chemother 66:1987–1991. https://doi.org/10.1093/jac/dkr225.
9. Phan MD, Kidgell C, Nair S, Holt KE, Turner AK, Hinds J, Butcher P, Cooke FJ, Thomson NR, Titball R, Bhutta ZA, Hasan R, Dougan G, Wain J. 2009. Variation in *Salmonella enterica* serovar typhi IncHI1 plasmids during the global spread of resistant typhoid fever. Antimicrob Agents Chemother 53:716–727. https://doi.org/10.1128/AAC.00645-08.
10. Villa L, García-Fernández A, Fortini D, Carattoli A. 2010. Replicon sequence typing of IncF plasmids carrying virulence and resistance determinants. J Antimicrob Chemother 65:2518–2529. https://doi.org/10.1093/jac/dkq347.
11. Argente M, Miró E, Martí C, Vilamala A, Alonso-Tarrés C, Ballester F, Calderón A, Gallés C, Gasós A, Mirelis B, Morta M, Olsina M, Sauca G, Sierra M, Rivera A, Navarro F. 2019. Molecular characterization of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* strains after a carbapenem resistance increase in Catalonia. Enferm Infecc Microbiol Clin 37:82–88. https://doi.org/10.1016/j.eimc.2018.02.003.
12. Burke L, Humphreys H, Fitzgerald-Hughes D. 2016. The molecular epidemiology of resistance in cefotaximase-producing Escherichia coli clinical isolates from Dublin, Ireland. Microb Drug Resist 22:552–558. https://doi.org/10.1089/mdr.2015.0154.
13. Ribeiro TG, Novais Â, Rodrigues C, Nascimento R, Freitas F, Machado E, Peixe L. 2019. Dynamics on clonal and plasmid backgrounds of Enterobacteriaceae producing acquired AmpC in Portuguese clinical settings throughout time. Int J Antimicrob Agents 53:650–656. https://doi.org/10.1016/j.ijantimicag.2019.03.013.
14. Xu L, Wang P, Cheng J, Qin S, Xie W. 2019. Characterization of a novel bla_{NDM-5}-harboring IncFII plasmid and an mcr-1-bearing IncI2 plasmid in a single *Escherichia coli* ST167 clinical isolate. Infect Drug Resist 12:511–519. https://doi.org/10.2147/IDR.S192998.
15. Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing: twenty-fourth informational supplement M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
16. Edelstein M, Pimkin M, Palagin I, Edelstein I, Stratchounski L. 2003. Prevalence and molecular epidemiology of CTX-M extended-spectrum β-lactamase-producing Escherichia coli and Klebsiella pneumoniae in Russian hospitals. Antimicrob Agents Chemother 47:3724–3732. https://doi.org/10.1128/aac.47.12.3724-3732.2003.
17. Lee SH, Kim JY, Lee SK, Jin W, Kang SG, Lee KJ. 2000. Discriminatory detection of extended-spectrum beta-lactamases by restriction fragment length dimorphism-polymerase chain reaction. Lett Appl Microbiol 31:307–312. https://doi.org/10.1046/j.1472-765x.2000.00806.x.
18. Ellington MJ, Kistler J, Livermore DM, Woodford N. 2007. Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases. J Antimicrob Chemother 59:321–322. https://doi.org/10.1093/jac/dkl481.
19. Espinal P, Miró E, Segura C, Gómez L, Plasencia V, Coll P, Navarro F. 2018. First description of bla_{NDM-7} carried on an IncX4 plasmid in *Escherichia coli* ST679 isolated in Spain. Microb Drug Resist 24:113–119. https://doi.org/10.1089/mdr.2017.0039.
20. Poirel L, Walsh TR, Cuvillier V, Nordmann P. 2011. Multiplex PCR for detection of acquired carbapenemase genes. Diagn Microbiol Infect Dis 70:119–123. https://doi.org/10.1016/j.diagmicrobio.2010.12.002.
21. Miró E, Mirelis B, Navarro F, Rivera A, Mesa RJ, Roig MC, Gómez L, Coll P. 2005. Surveillance of extended-spectrum β-lactamases from clinical samples and faecal carriers in Barcelona, Spain. J Antimicrob Chemother 56:1152–1155. https://doi.org/10.1093/jac/dki395.
22. Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. 2016. Fecal colonization with extended-spectrum beta-lactamase-producing Enterobacteriaceae and risk factors among healthy individuals: a systematic review and metaanalysis. Clin Infect Dis 63:310–318. https://doi.org/10.1093/cid/ciw283.
23. Carattoli A, Seiffert SN, Schwendener S, Perreten V, Endimiani A. 2015. Differentiation of IncL and IncM plasmids associated with the spread of clinically relevant antimicrobial resistance. PLoS One 10:e0123063. https://doi.org/10.1371/journal.pone.0123063.
24. García-Fernández A, Fortini D, Veldman K, Mevius D, Carattoli A. 2009. Characterization of plasmids harbouring qnrS1, qnrB2 and qnrB19 genes in *Salmonella*. J Antimicrob Chemother 63:274–281. https://doi.org/10.1093/jac/dkn470.
25. Johnson TJ, Bielak EM, Fortini D, Hansen LH, Hasman H, Debroy C, Nolan LK, Carattoli A. 2012. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. Plasmid 68:43–50. https://doi.org/10.1016/j.plasmid.2012.03.001.
26. Weingarten RA, Johnson RC, Conlan S, Ramsburg AM, Dekker JP, Lau AF, Khil P, Odom RT, Deming C, Park M, Thomas PJ, Henderson DK, Palmore TN, Segre JA, Frank KM. 2018. Genomic analysis of hospital plumbing reveals diverse reservoir of bacterial plasmids conferring carbapenem resistance. mBio 9:e02011-17. https://doi.org/10.1128/mBio.02011-17.
27. Johnson TJ, Wannemuehler YM, Johnson SJ, Logue CM, White DG, Doetkott C, Nolan LK. 2007. Plasmid replicon typing of commensal and pathogenic *Escherichia coli* isolates. Appl Environ Microbiol 73:1976–1983. https://doi.org/10.1128/AEM.02171-06.
28. Irrgang A, Falgenhauer L, Fischer J, Ghosh H, Guiral E, Guerra B, Schmoger S, Imirzalioglu C, Chakraborty T, Hammerl JA, Käsbohrer A. 2017. CTX-M-15-producing *E. coli* isolates from food products in Germany are mainly associated with an IncF-type plasmid and belong to two predominant clonal *E. coli* lineages. Front Microbiol 8:2318. https://doi.org/10.3389/fmicb.2017.02318.
29. Zhao WH, Hu ZQ. 2013. Epidemiology and genetics of CTX-M extended-spectrum β-lactamases in Gram-negative bacteria. Crit Rev Microbiol 39:79–101. https://doi.org/10.3109/1040841X.2012.691460.
30. Dupouy V, Abdelli M, Moyano G, Arpaillange N, Bibbal D, Cadiergues MC, Lopez-Pulin D, Sayah-Jeanne S, De Gunzburg J, Saint-Lu N, Gonzalez-Zorn B, Andremont A, Bousquet-Mélou A. 2019. Prevalence of beta-lactam and quinolone/fluoroquinolone resistance in Enterobacteriaceae from dogs in France and Spain—characterization of ESBL/pAmpC isolates, genes, and conjugative plasmids. Front Vet Sci 6:279. https://doi.org/10.3389/fvets.2019.00279.
31. Pietsch M, RESET Study Group, Irrgang A, Roschanski N, Brenner Michael G, Hamprecht A, Rieber H, Käsbohrer A, Schwarz S, Rösler U, Kreienbrock L, Pfeifer Y, Fuchs S, Werner G, Bühling A, Domurath B, Wendt C, Valenza G, Wahl HG, Hellkamp J, Arvand M, Kresken M, Podschun R, Schneider S, Tobisch S, Witt M, Eckmanns T, Hachmann U, Bührlen U, von Salviati-Claudius C, Lu HM, Laube H, Hering J. 2018. Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. BMC Genomics 19:601. https://doi.org/10.1186/s12864-018-4976-3.
32. Robin F, Beyrouthy R, Bonacorsi S, Aissa N, Bret L, Brieu N, Cattoir V, Chapuis A, Chardon H, Degand N, Dubois V, Fortineau N, Grillon A, Lanotte P, Leyssene D, Patry I, Podglajen I, Recule C, Ros A, Ponties V, Ploy MC, Bonnet R. 2017. Inventory of extended-spectrum-β-lactamase-producing Enterobacteriaceae in France as assessed by a multicenter study. Antimicrob Agents Chemother 61:e01911-16. https://doi.org/10.1128/AAC.01911-16.
33. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for detecting and sizing large plasmids. Anal Biochem 226:235–240. https://doi.org/10.1006/abio.1995.1220.
34. Branger C, Ledda A, Billard-Pomares T, Doublet B, Fouteau S, Barbe V, Roche D, Cruveiller S, Médigue C, Castellanos M, Decré D, Drieux-Rouze L, Clermont O, Glodt J, Tenaillon O, Cloeckaert A, Arlet G, Denamur E. 2018. Extended-spectrum beta-lactamase-encoding genes are spreading on a wide range of *Escherichia coli* plasmids existing prior to the use of third-generation cephalosporins. Microb Genom 4:e000203. https://doi.org/10.1099/mgen.0.000203.
35. Moran RA, Anantham S, Pinyon JL, Hall RM. 2015. Plasmids in antibiotic susceptible and antibiotic resistant commensal *Escherichia coli* from healthy Australian adults. Plasmid 80:24–31. https://doi.org/10.1016/j.plasmid.2015.03.005.
36. Carattoli A, Villa L, Fortini D, García-Fernández A. 5 December 2018. Contemporary IncI1 plasmids involved in the transmission and spread of antimicrobial resistance in Enterobacteriaceae. Plasmid https://doi.org/10.1016/j.plasmid.2018.12.001.
37. Rozwandowicz M, Brouwer MSM, Fischer J, Wagenaar JA, Gonzalez-Zorn B, Guerra B, Mevius DJ, Hordijk J. 2018. Plasmids carrying antimicrobial resistance genes in Enterobacteriaceae. J Antimicrob Chemother 73:1121–1137. https://doi.org/10.1093/jac/dkx488.

38. Smith H, Bossers A, Harders F, Wu G, Woodford N, Schwarz S, Guerra B, Rodríguez I, van Essen-Zandbergen A, Brouwer M, Mevius D. 2015. Characterization of epidemic IncI1-Iγ plasmids harboring Ambler class A and C genes in *Escherichia coli* and *Salmonella enterica* from animals and humans. Antimicrob Agents Chemother 59:5357–5365. https://doi.org/10.1128/AAC.05006-14.
39. Yang QE, Sun J, Li L, Deng H, Liu BT, Fang LX, Liao XP, Liu YH. 2015. IncF plasmid diversity in multi-drug resistant *Escherichia coli* strains from animals in China. Front Microbiol 6:964. https://doi.org/10.3389/fmicb.2015.00964.
40. Matsumura Y, Kyoto-Shiga Clinical Microbiology Study Group, Yamamoto M, Nagao M, Ito Y, Takakura S, Ichiyama S. 2013. Association of fluoroquinolone resistance, virulence genes, and IncF plasmids with extended-spectrum-β-lactamase-producing Escherichia coli sequence type 131 (ST131) and ST405 clonal groups. Antimicrob Agents Chemother 57:4736–4742. https://doi.org/10.1128/AAC.00641-13.
41. Ho WS, Yap KP, Yeo CC, Rajasekaram G, Thong KL. 2016. The complete sequence and comparative analysis of a multidrug-resistance and virulence multireplicon IncFII plasmid pEC302/04 from an extraintestinal pathogenic *Escherichia coli* EC302/04 indicate extensive diversity of IncFII plasmids. Front Microbiol 6:1547. https://doi.org/10.3389/fmicb.2015.01547.
42. Ghosh H, Doijad S, Falgenhauer L, Fritzenwanker M, Imirzalioglu C, Chakraborty T. 2017. *bla*CTX-M-27-encoding *Escherichia coli* sequence type 131 lineage C1-M27 clone in clinical isolates. Emerg Infect Dis 23:1754–1756. https://doi.org/10.3201/eid2310.170938.
43. Cantón R, González-Alba JM, Galán JC. 2012. CTX-M enzymes: origin and diffusion. Front Microbiol 3:110. https://doi.org/10.3389/fmicb.2012.00110.
44. Clermont O, Christenson JK, Denamur E, Gordon DM. 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ Microbiol Rep 5:58–65. https://doi.org/10.1111/1758-2229.12019.
45. Johnson JR, Stell AL. 2000. Extended virulence genotypes of *Escherichia coli* strains from patients with urosepsis in relation to phylogeny and host compromise. J Infect Dis 181:261–272. https://doi.org/10.1086/315217.
46. Oteo J, Spanish Network for Research in Infectious Diseases (REIPI), González-López JJ, Ortega A, Quintero-Zárate JN, Bou G, Cercenado E, Conejo MC, Martínez-Martínez L, Navarro F, Oliver A, Bartolomé RM, Campos J. 2014. Inhibitor-resistant TEM- And OXA-1-producing *Escherichia coli* isolates resistant to amoxicillin-clavulanate are more clonal and possess lower virulence gene content than susceptible clinical isolates. Antimicrob Agents Chemother 58:3874–3881. https://doi.org/10.1128/AAC.02738-13.
47. Tenaillon O, Skurnik D, Picard B, Denamur E. 2010. The population genetics of commensal *Escherichia coli*. Nat Rev Microbiol 8:207–217. https://doi.org/10.1038/nrmicro2298.
48. Johnson TJ, Nolan LK. 2009. Pathogenomics of the virulence plasmids of *Escherichia coli*. Microbiol Mol Biol Rev 73:750–774. https://doi.org/10.1128/MMBR.00015-09.